29th April, 1965.

Dr. R.W. Holley,
Dept. of Biochemistry,
Cornell University,
Ithaca, New York,
U.S.A.

Dear Dr. Holley,

Vernon Ingram gave me an early copy of your Abstract and I have just read your recent paper in Nature. I must really congratulate you on a magnificent piece of work. Like everybody else I have been concerned to spot the anti-codon. For a variety of reasons I think it must be IGC. Not only is the molecule split in high Mg by both RNase and T, in their region, but more compellingly Ingram finds the sequence IAC in valine S-RNA, and Zachau the sequence IGA in serine S-RNA. These are exactly what one would predict from our knowledge of the genetic code (I enclose a copy of the best version known to me at the present).

Of course it is possible that IGC is the anti-codon for GCU, GCC, GCA and GCG. For various reasons I feel that this is not very likely. I think it very probable that it is the anti-codon for both GCU and GCC. This is because I have noticed that the base-pair I = U (or G = U) is very similar to the two usual base-pairs. A little bit of wobble in the third place would then give the ambiguity, and allow one S-RNA molecule to recognise two different triplets.

I asked Nirenberg if he were testing the binding properties of your S-RNA, but he tells me that you yourself are doing it. I know I don't have to tell you how important it is to do this. However, binding studies using trinucleotides are not completely reliable, especially at high Mg and low temperature, as Nirenberg will tell you, so it may be difficult to get a clean answer.

I have asked myself, assuming that IGC is the anti-codon only for GCU and GCC, what anti-codons we might expect for GCA and GCG, assuming that both code for alanine (it seems certain that at least one of them must). The obvious answers are UGC and CGC, but I should not be surprised if UGC was the anti-codon for both GCA and GCG, again by using a U = G pair and allowing some wobble? This makes me wonder whether you are certain that your S-RNA is completely pure, or whether it may be a mixture of two very closely related species, one having IGC and the other having UGC in its place. Would you have been able to detect this? Of course they might not have been present in equal amounts.

I'm sure you must be working on all these problems, but I thought you might like to know of the possibility of an I = U (or G = U) pair in the third place in the codon. I would guess that the base-pairing in the first two places in the codon is likely to be of the standard type found in DNA.

Once again many congratulations to you and your colleagues on your achievement. I hope to be at the Gordon Conference on Nucleic Acids this year and hope I shall have the pleasure of meeting you again there.

With all good wishes,

Yours sincerely,

F. H. C. Crick